

## Bacterial community composition and richness in biofilms of the Yasu and Ado Rivers

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Biofilm bacteria play important roles in the biogeochemical cycling of river ecosystems through processes such as accumulation, decomposition, and assimilation of organic matter. Although bacterial community compositions (BCCs) have previously been reported, factors determining their spatial distribution patterns are still poorly understood. It is difficult to disentangle confounding factors affecting the BCCs in stream/river biofilms due to the high spatial correlation among environmental variables within stream networks. In this study, we focused on BCC variations within and between tributaries of two rivers which have different land use patterns in their catchments in order to evaluate the relative importance of geographical and local habitat variables on BCCs.

Samples were collected from several tributaries in the Yasu and Ado Rivers draining into the Lake Biwa, Japan. Five stones were collected at each site and biofilm was detached from 6 cm square surface of each stone for DNA extraction. The extracted DNA was sequenced on Illumina MiSeq and clustered into operational taxonomic units (OTUs) at a 97 % sequence similarity level. Environmental parameters were measured and separated into two categories: geographical variables (altitude, catchment area, and land use) and local habitat variables (river depth, current velocity, water temperature, canopy openness, electric conductivity, total nitrogen, and total phosphorus).

8,547 OTUs were obtained after rarefying reads to the lowest coverage of reads and *Bacteroidetes*, *Alphaproteobacteria*, *Betaproteobacteria* and *Cyanobacteria*, all of which has been often regarded as common taxa in river biofilms, dominated in both rivers. BCCs were significantly different at the catchment scale between Yasu and Ado Rivers (PERMANOVA,  $p < 0.001$ ). When the data from both rivers were pooled, two local habitat factors, water temperature and electric conductivity, significantly accounted for the dissimilarity of BCCs in both rivers (Mantel test,  $p < 0.001$ ). However, when the data were analyzed separately between the two rivers, neither geographical nor local habitat factors significantly determined the spatial variation of BCCs within the river network system (PERMANOVA,  $p > 0.05$  and Mantel test,  $p > 0.05$ ). In both rivers, bacterial richness decreased with increasing current velocity (Spearman rank test,  $p < 0.01$ ).

Our data suggest that (1) BCCs across Yasu and Ado River watersheds are more influenced by local habitat factors than by geographic factors; and (2) physical disturbance by high current velocity could be

a primary factor affecting bacterial diversity in biofilms of river ecosystems.

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